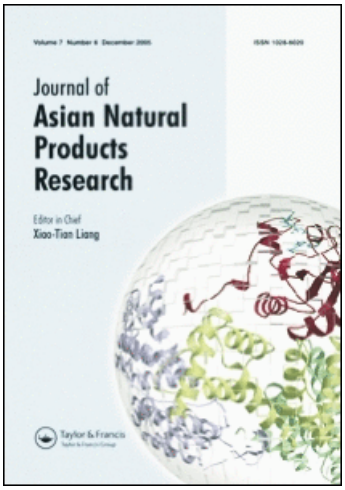

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Synthesis, structure, and bioevaluation of 2,5-bis(arylmethenyl)cyclopentanones

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To cite this Article Liang, Guang , Yang, Shu-Lin , Shao, Li-Li , Zhao, Cheng-Guang , Xiao, Jian , Lv, Yan-Xia , Yang, Ju , Zhao, Yu and Li, Xiao-Kun(2008) 'Synthesis, structure, and bioevaluation of 2,5-bis(arylmethenyl)cyclopentanones', Journal of Asian Natural Products Research, 10: 10, 957 — 965
To link to this Article: DOI: 10.1080/10286020802181257
URL: http://dx.doi.org/10.1080/10286020802181257

Journal of Asian Natural Products Research
Vol. 10, No. 10, October 2008, 957–964

Synthesis, structure, and bioevaluation of 2,5-bis(arylmethenyl)cyclopentanones

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(*Received 8 January 2008; final version received 30 April 2008*)

Curcumin is an excellent lead compound with a variety of bioactivity. Recent articles reported that curcumin's instability and low bioavailability *in vivo* are mainly due to its easily decomposable β-diketone moiety. With the aim of bioactive curcumin analogs with better pharmacokinetic property, we present here 11 bis(arylmethenyl)cyclopentanones similar to curcumin and without β-diketone moiety by reacting relevant arylaldehydes and cyclopentanones. The analogs were structurally determined by ^{1}HNMR and MS spectra, and the crystal structure of **6** was analyzed by X-ray diffraction. Their antibacterial activities *in vitro* against seven Gram-positive and Gram-negative bacteria were tested, and their inhibition of TNF-α and IL-6 secretion in LPS-induced mouse macrophages was investigated using enzyme-linked immunosorbent assay. It was observed that several derivatives displayed higher activity when compared with curcumin, and most of the analogs exhibited activities against the ampicillin-resistant Gram-negative *Enterobacter cloacae*.

Keywords: curcumin analogs; synthesis; crystal structure; antibacteria; antiinflammation

1. Introduction

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a main bioactive component isolated from the roots of *Curcuma Longa* Chen., has thousands of years of history as a spice and traditional medicine. In the past decades, many studies have showed that curcumin possesses a variety of bioactivity, such as antibacterial, antiinflammation, antioxidation, antimutation, decreasing serum cholesterol content and atherosclerosis, and antitumor [1–5]. An increasing amount of attention has been paid to the use of curcumin in antiinflammation. Curcumin functions by inhibiting the release of certain kinds of cytokines. It inhibits the activation of transcriptional factor NF-κB and Iκ-Bα in macrophages, and subsequently suppresses the expression of tumor necrosis factor (TNF-α), IL-6, and COX-2 to alleviate the inflammatory responses [6,7]. Curcumin is under the phase II clinical trial.

Unfortunately, curcumin has some disadvantages in pharmacokinetics, for example, unstable metabolism *in vivo*, ease to decompose in a neutral and basic condition, and low bioavailability. In the phase I clinical trial, curcumin concentration in serum and urine reached only 11.1 nmol/l and 1.3 μmol/l, respectively, after an oral administration of 3600 mg of curcumin per day [8,9]. Most recent researches demonstrated that the main cause of the decomposition *in vivo* was the non-cytochrome P450 pathway, by which

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ISSN 1028-6020 print/ISSN 1477-2213 online

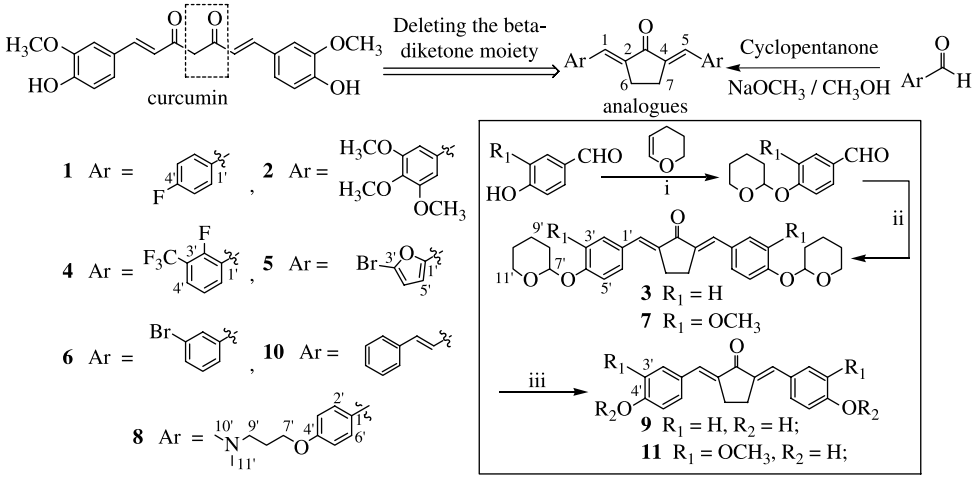
DOI: 10.1080/10286020802181257
http://www.informaworld.com

the β-diketone moiety of curcumin can be decomposed quickly [10]. Therefore, a kind of curcumin analog without the β-diketone moiety may possess a better property in pharmacokinetics. In this paper, we present 11 2,5-bis(arylmethenyl)cyclopentanones using cyclopentanone as the linking group instead of the β-diketone group, and then evaluate their activity in inhibiting LPS-induced secretion of pro-inflammatory factors in macrophages using enzyme-linked immunosorbent assay (ELISA) and suppressing the growth of several bacteria. Our results showed that several derivatives displayed higher anti-inflammatory activity when compared with curcumin and most of the analogs exhibited activity against the ampicillin-resistant Gram-negative *Enterobacter cloacae*. Especially, compound **8** with a long-strain substitution showed marked activities against both bacteria and endotoxin-induced inflammation, indicating that it cannot only inhibit the inflammation induced by bacterial endotoxin but can also eliminate the pathogen directly.

2. Results and discussion

The structure and general synthesis of analogs designed are shown in Scheme 1. Compounds **1**, **2**, **4**, **5**, **6**, **8**, and **10** were synthesized by coupling the appropriate aromatic aldehyde with cyclopentanone in an alkaline condition. For the synthesis of **9** and **11**, 4-hydroxybenzaldehyde and 3-methoxy-4-hydroxybenzaldehyde were first reacted with the tetrahydropyran-2-yl to get protective derivatives and then **3** and **7** through a coupling with cyclopentanone. Compound **9** or **11** was subsequently obtained by hydrolyzation of **3** or **7**, respectively, with a catalytic amount of *p*-toluenesulfonic acid. The ^{1}HNMR evidence that there did not appear the peaks of methylene adjacent to the carbonyl group in cyclopentanone analogs confirmed the absence of mono-coupled compounds with single aryl ring.

An X-ray crystal structure and atom-labeling scheme of the analog **6**, (2*E*,5*E*)-2,5-bis(3-bromobenzylidene)cyclopentanone, is shown in Figure 1. Compound **6** is completely symmetrical and possesses a more rigid structure than curcumin, due to the introduction of cyclopentanone. All the non-hydrogen atoms are coplanar in mono-carbonyl curcumin analog **6**, while the dihedral angle between the least-square planes is 19.1(3)° [11]. H atoms bound to C atoms were placed in idealized positions, with C—H = 0.93 Å



Scheme 1. Chemical structures and general synthesis of curcumin analogs. Reagents and conditions: (i) pyridine–PTSA, CH_2Cl_2, rt; (ii) Cyclopentanone, NaOH/EtOH, rt; (iii) PTSA, MeOH, rt.

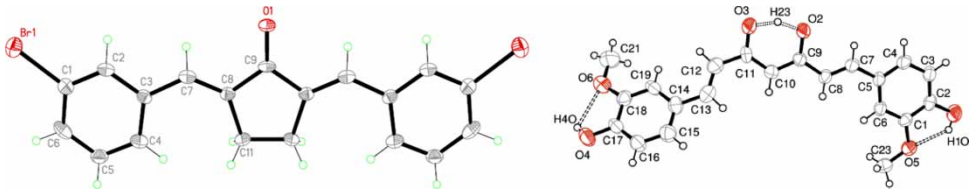


Figure 1. The crystal structure of **6** (left) and curcumin (right) [11] with labeled non-hydrogen atom, showing the displacement ellipsoids at the 50% probability level. Dashed lines indicate intramolecular

Table 1. Selected bond lengths (Å).

Bond	Distance	Bond	Distance	Bond	Distance
Br(1)–C(1)	1.893(8)	C(2)–C(3)	1.397(10)	C(7)–C(8)	1.324(10)
C(1)–C(2)	1.368(11)	C(3)–C(7)	1.465(10)	C(8)–C(9)	1.463(9)
C(9)–O(1)	1.240(11)	C(8)–C(11)	1.523(9)	C(11)–C(11′)	1.515(15)

hydrogen bonding and vinyl H or 0.96 Å for methylene H. Of course, there are no hydrogen bonds in **6**. The distance from Br(1) to Br(2) representing the molecular length of **6** is 15.194 Å, while that of curcumin is 16.910 Å from *O*(1) to *O*(4) [11], probably indicating the difference in the bioactivity of them. The selected bond lengths are listed in Table 1.

As shown in Figure 2, a majority of curcumin analogs displayed activity against the release of TNF-α and IL-6 induced by LPS in different degrees. The inhibiting rate of compounds **1**, **3**–**8**, and **10** against LPS-induced TNF-α release varied from 70 to 45% and **10** exhibited the highest activity against TNF-α release with inhibiting rate of 45.1%. Compound **10** also showed a high activity against LPS-induced IL-6 release (inhibiting rate 19.7%). The highest activity inhibiting IL-6 release appeared in compound **8**, which almost reached a complete block to IL-6 release (3.5%). Compounds **9** and **11** with hydroxyl group as curcumin displayed, however, completely different activity. Compound **11** was slightly stronger than curcumin against LPS induction, while **9** played a role to enhance the LPS-induced release of both TNF-α and IL-6.

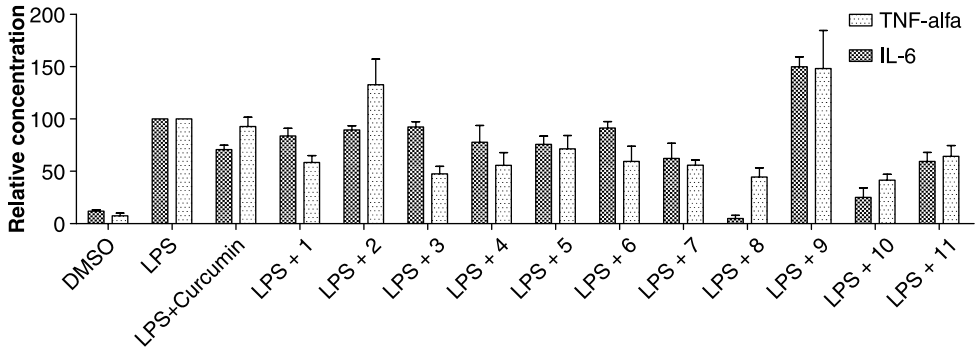


Figure 2. Inhibition of LPS-induced TNF-α and IL-6 by curcumin and its analogs in J774A.1 macrophages. Cells were pretreated with curcumin or its analogs (10 μM) for 3 h, and then treated with LPS (0.5 μg/ml) for 21 h. TNF-α and IL-6 levels in the culture media were measured by ELISA. The results were expressed the percent of LPS control. Each bar represents mean ± SE of three independent experiments.

The activities of these compounds against bacterial growth are shown in Table 2 and ampicillin was used as a positive control. A part of compounds displayed higher activities against bacterial growth than curcumin. Compound **8**, a novel compound with a long-chain substitute of 3-(dimethylamino)propoxyl, exhibited bioactivity against all the seven bacteria tested and possessed inhibiting property against *Staphylococcus epidermidis* as strongly as ampicillin does, indicating that the long-chain substitute may play a role to enhance the antibacterial activity. Heterocycle-substituted **5**, long-conjugated **10**, and hydroxybenyl-containing **11** exhibited the high activity against Gram-positive bacteria, but no activity against Gram-negative *Escherichia coli*. More importantly, these 11 compounds and leading curcumin all exhibited inhibiting activity against ampicillin-resistant Gram-negative *E. cloacae*, indicating that they may appear to have different antibacterial mechanism from ampicillin.

The difference between **9** and **11**, which showed opposite properties in ELISA and different activity against bacteria growth, suggested that the 3′-methoxyl may play a significant role in the activity of analogs with 4′-hydroxyl substitute.

3. Conclusion

In this paper, we present the synthesis, antibacterial, and antiinflammatory evaluation of 11 mono-carbonyl curcumins without the β-diketone moiety. The endotoxin from a pathogen, including bacteria, is a significant factor resulting in cellular inflammatory response and immune disease generation, and macrophages play a critical role in both initiation and progression of inflammation. The LPS from the wall of Gram-negative bacteria can induce inflammatory stress by activating a variety of signal pathway and pro-inflammatory factors (e.g. TNF-α and IL-6) [12]. Our results show that curcumin analog **8** is able to not only inhibit the release of TNF-α and IL-6 induced by bacterial endotoxin but also suppress directly the growth of pathogen. However, the underlying pharmacological mechanisms of curcumin and its derivatives remain unknown and are the focus of our current research. These new compounds would be useful for the development of new antiinflammatory drugs.

4. Experimental

4.1 General experimental procedures

Melting points were determined on a Fisher–Johns melting apparatus and are uncorrected. ^{1}HNMR spectra were recorded on a Varian INOVA-400 spectrometer. Electron-spray ionization mass spectra (ESIMS) in positive mode were recorded on a Bruker Esquire 3000^+ spectrometer. Column chromatography purifications were carried out on silica gel 60 (E. Merck, 70–230 mesh). The purity of all new compounds was checked using thin-layer chromatography (TLC) and ^{1}HNMR spectra. All reagents are business available from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), and are of analytical grade.

4.2 General procedure of 1, 2, 4, 5, 6, 8, and 10

To a solution of 10 mmol arylaldehyde (5-bromofuranaldehyde for **5** and cinnamaldehyde for **10**) in dried EtOH (10 ml), 5 mmol cyclopentanone was added. The solution was stirred at room temperature for 10 min, followed by the dropwise addition of 18%(w/v) $NaOCH_3$ (1.5 ml, 5 mmol). The mixture was stirred at room temperature and monitored with TLC. When the reaction completed, the residue was poured into cold saturated NH_4Cl solution and filtered. The precipitate was washed with water, cold ethanol, and then cold acetone and dried in vacuum. The solid was purified by chromatography over silica gel using CH_2Cl_2/CH_3 OH as the eluent to yield compounds **1**, **2**, **4**, **5**, **6**, **8**, and **10** [13].

4.2.1 Compound 1

Yellow crystal of **1** was obtained by recrystallization from ethanol/water (9:1),

Table 2. Antibacterial activity of curcumin analogs against bacterial strains in different dilutions (10, 5, and 2.5 mM).

	Gram-positive					Gram-negative	
Compound	*S. aureus*	*Micrococcus*	*S. saprophyticus*	*S. epidermidis*	*Enterococcus* sp.	*E. cloacae*	*E. coli*
1	–	–	–	–	–	10,9, −	9, − , −
2	–	10, − , −	–	–	9, − , −	10, − , −	–
3	–	–	–	–	–	13,9, −	–
4	–	–	–	–	–	11, − , −	–
5	12,9, −	10, − , −	10, − , −	9, − , −	–	10,9, −	–
6	–	12,10, −	–	–	–	10, − , −	–
7	–	–	–	–	–	11, − , −	–
8	20,15,13	17,17,14	17,14,12	18,16,14	17,15,14	13,12,10	14,14,13
9	–	12, − , −	–	–	–	10, − , −	–
10	12,9, −	14, − , −	–	10,8, −	–	12, − , −	–
11	12, − , −	12, − , −	10, − , −	10, − , −	–	11,10,9	–
Curcumin	11,10, −	9, − , −	10,10, −	11,9, −	10,10, −	11,11, −	10,10, −
Ampicillin	30,26,20	23,18, −	18,16, −	18,14,10	23,20,16	–	31,26,24

Numerals show size of zone of inhibition in mm; the size (mm) of pores on agar is 4 mm.

88% yield, m.p. 239–240°C: ^{1}HNMR ($CDCl_3$): δ (ppm) 3.10 (4H, s, 6,7-H), 7.14 (4H, t, $J = 8.4$ Hz, 2′,6′-H), 7.59 (4H, t, $J = 9.0$ Hz, 3′,5′-H), 7.61 (2H, s, 1,5-H). ESIMS *m/z*: 297.13 $[M + 1]^+$.

4.2.2 *Compound* ***2***

Yellow powder, 90% yield, m.p. 202–204°C (204°C) [14].

4.2.3 *Compound* ***4***

Yellow powder, 91% yield, m.p. 223°C: ^{1}HNMR($CDCl_3$): δ (ppm) 3.05 (4H, s, 6,7-H), 7.32 (2H, t, $J = 8.0$ Hz, 5′-H), 7.64 (2H, t, $J = 7.0$ Hz, 6′-H), 7.75 (2H, t, $J = 7.0$ Hz, 4′-H), 7.80 (2H, s, 1,5-H). ESIMS *m/z*: 430.92 $[M - 1]^-$.

4.2.4 *Compound* ***5***

Yellow powder, 77% yield, m.p. 176°C: ^{1}HNMR($CDCl_3$): δ (ppm) 3.06 (4H, s, 6,7-H), 6.47 (2H, d, $J = 3.6$ Hz, 4′-H), 6.65 (2H, d, $J = 3.6$ Hz, 5′-H), 7.25 (2H, s, 1,5-H). ESIMS *m/z*: 398.81 $[M + 1]^+$.

4.2.5 *Compound* ***6***

Yellow powder, 91% yield, m.p. 186°C (188–200°C) [15].

4.2.6 *Compound* ***8***

Yellow powder, 72% yield, m.p. 154°C: ^{1}HNMR($CDCl_3$): δ (ppm) 1.98 (4H, m, 8′-H), 2.26 (12H, s, 10′,11′-CH_3), 2.46 (4H, t, $J = 7.4$ Hz, 9′-H), 3.08 (4H, s, 6,7-H), 4.07 (4H, t, $J = 6.2$ Hz, 7′-H), 6.96 (4H, d, $J = 8.8$ Hz, 2′,6′-H), 7.73 (2H, s, 1,5-H), 7.56 (4H, d, $J = 8.8$ Hz, 3′,5′-H × 2). ESIMS *m/z*: 463.33 $[M + 1]^+$.

4.2.7 *Compound* ***10***

Yellow powder, 63% yield, m.p. 229°C (223–224°C) [16].

4.3 *General procedure of 3 and 7*

A solution of 3,4-dihydro-α-pyran (0.2 mol) in dichloromethane (10 ml) was added dropwise onto a well-stirred suspension of hydroxyl benzaldehyde (0.1 mol) and a catalytic amount of pyridinium *p*-toluenesulfonate in the same solvent (40 ml). The mixture was stirred at room temperature for 3 h and monitored by TLC, then washed with cold water, saturated $NaHCO_3$ solution (50 ml × 3), and brine (50 ml × 3) and dried over Na_2SO_4. Evaporation of the solvent gave crude product that was purified by column chromatography on silica gel (hexane/ethyl acetate, 8:2, as eluent) to obtain the mediate 4-(tetrahydropyran-2-yloxy)benzaldehyde as a lightly yellow oil (69–78% yield). A mixture of protected derivative (10 mmol) and cyclopentanone (5 mmol) in C_2H_5OH (10 ml) was stirred at room temperature for 10 min, and then 18% (w/v) $NaOCH_3$ (1.5 ml, 5 mmol) was added dropwise. After stirring for 2 h, the mixture was treated with 100 ml cold water and filtered. The residue was washed with ethanol and acetone and dried in vacuum. The product was purified by recrystallization from ethanol/water (9:1) to obtain **3** and **7**.

4.3.1 *Compound* ***3***

Yellow powder, 72% yield, m.p. 209°C: ^{1}HNMR($CDCl_3$): δ (ppm) 1.60–1.90 (12H, m, 8′,9′,10′-H), 3.09 (4H, s, 6,7-H), 3.61 (2H, q, $J = 5.2$ Hz, 11′-H^e × 2), 3.90 (2H, q, $J = 10.7$ Hz, 11′-H^a), 5.50 (2H, t, 7′-H), 7.11 (4H, m, $J = 8.8$ Hz, 2′,6′-H), 7.56 (6H, m, 1,5,3′,5′-H). ESIMS *m/z*: 460.92 $[M]^+$.

4.3.2 *Compound* ***7***

Yellow powder, 67% yield, m.p. 138°C: ^{1}HNMR($CDCl_3$): δ (ppm) 1.70–1.95 (12H, m, 8′,9′,10′-H), 3.11 (4H, s, 6,7-H), 3.63 (2H, q, $J = 7.2$ Hz, 11′-H^e × 2), 3.90 (2H, q, $J = 11.6$ Hz, 11′-H^a), 3.91 (6H, s, 12′-H), 5.50 (2H, s, 7′-H), 7.09–7.23 (6H, m, Ar-H), 7.54 (2H, s, 1,5-H). ESIMS *m/z*: 520.86 $[M]^+$.

4.4 *General procedure of 9 and 11*

The protected derivatives (**3** or **7**, 10 mmol) were suspended in ethanol (20 ml) and treated

with a catalytic amount of *p*-toluenesulfonic acid. After stirring at room temperature for 10 h, 100 ml cold water was added and the mixture was filtered. The residue was washed with cold water and then recrystallized from C_2H_5OH/H_2O (9:1) to obtain pure **9** or **11**.

4.4.1 Compound **9**

Yellow crystal, 89% yield, mp > 300°C: ^{1}HNMR(DMSO-d_6): δ (ppm): 3.01 (4H, s, 6,7-H), 6.88 (4H, d, $J = 8.4$ Hz, 2′,6′-H), 7.33 (2H, s, 1,5-H), 7.53 (4H, d, $J = 8.4$ Hz, 3′,5′-H), 10.01 (2H, brs, OH). ESIMS *m/z*: 291.13 $[M - 1]^-$.

4.4.2 Compound **11**

Yellow crystal, 76% yield, m.p. 214°C (212–214°C) [17].

4.5 X-ray crystallography

A yellow rhombus crystal of $C_{19}H_{14}Br_2O$ (6) from CH_3OH/CH_2Cl_2 (7:3) having approximate dimensions of 0.25 × 0.21 × 0.08 mm was mounted on a glass fiber. All measurements were made on a Bruker X8 APEX diffractometer with graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) and a $\varphi-\omega$ scan mode. Of the 7218 reflections that were collected in the range of $1.2 < \theta < 25.0°$, 1429 were unique ($R_{int} = 0.099$). Data were collected and integrated using the Bruker SAINT [18] software package. All non-hydrogen atoms were refined anisotropically [19]. The hydrogen atoms were determined with theoretical calculations. A full-matrix least-squares refinement gave the final $R = 0.062$ and $wR = 0.187 (w = 1/[\sigma^2(F_o^2) + (0.1007P)^2 + 0.0000P])$, where $P = (F_o^2 + 2F_c^2)/3$, $(\Delta/\sigma)\text{max} < 0.001$, $S = 1.03$, $(\Delta\rho)\text{max} = 0.54$ e/Å^{-3}, and $(\Delta\rho)\text{min} = -0.98$ e/Å^{-3}. All refinements were performed using the SHELXTL-97 crystallographic software package [20]. The results showed that the crystal belongs to the monoclinic system, space group $-P2$ with $a = 6.225(5)$ Å, $b = 35.40(3)$ Å, $c = 7.199(5)$ Å, $\alpha = \beta = \gamma = 90.0°$, $V = 1586(2)$ Å^3, $Z = 4$, $F_{000} = 824$, $Dx = 1.751$ g/cm^{-3}, and $\mu = 5.11$ mm^{-1}.

4.6 Antiinflammatory test by ELISA

The mouse J774A.1 cells in our experiments belong to macrophage-like cell line and are widely used as a kind of cell model *in vitro* for inflammatory research. J774A.1 cells were kindly presented by Dr HP Zhou from Virginia Commonwealth University Medical School. Cells were plated in DMEM medium (including 10% FBS, 100 μg/ml penicillin, and 100 μg/ml streptomycin) with a density of 1.2×10^6/plate overnight at 37°C and in 5% CO_2. Cells were then pretreated with 10 μM of curcumin, each analog or vehicle control for 3 h, followed by treatment with LPS (0.5 μg/ml) for 21 h. At the end of the treatment, the culture media and the cells were collected. The levels of TNF-α and interleukin-6 (IL-6) in the media were determined by ELISA using mouse TNF-α and mouse IL-6 ELISA Max™ Set Deluxe Kits (Biolegend, USA). The tests were performed according to the manufacturer's instruction. The cells collected were added to the total lysis buffer (Tris–HCl, 20 mM; NP40, 1%; NaCl, 150 mM; EDTA, 2 mM; SDS, 0.1%; NaF, 20 mM; Na_3VO_4, 20 mM; H_2O) and vortexed, and then centrifuged at 8000 rpm for 5 min to extract the total proteins. The total protein concentrations of the viable cell pellets were determined using Bio-Rad (Hercules, CA, USA) protein assay reagents. Total amounts of the TNF-α and IL-6 in the media were normalized to the total protein amount of the viable cell pellets. Each compound was repeatedly tested in three independent experiments using macrophages with different generation [21].

4.7 Antibacterial evaluation

The antibacterial susceptibility test was done by determining the zone of inhibition. The curcumin analogs were weighed and dissolved in DMSO to make a solution of 10 mM concentration. From this stock solution, serial

dilutions have been done to 5, 2.5, 1.25, and 0.625 mM with DMSO in sterile test tubes. Seven different bacteria were selected viz. *Staphylococcus aureus* 26112 (ATCC 25923), *Micrococcus luteus* 28001, *Staphylococcus saprophyticus* 3–87, *S. epidermidis* 26069, *Enterococcus* sp. 050901, *Enterobacter cloacae* 45301, and *E.coli* 2765, which were obtained from the diagnosed patients from the First Affiliated Hospital of Wenzhou Medical College without antibiotic for testing isolates. Then they were subcultured on Sabourad dextrose agar slant and incubated at 37°C for 10–12 days. LB solid medium (prepared by dissolving 5 g yeast extract, 10 g tryptone, 5 g NaCl, and 15 g powdered agar in deionized water, metered volume to 1000 ml, autoclaved, and then kept under 4°C) was autoclaved and cooled to about 40°C, then an overnight culture of bacteria was added and intensively mixed. The bacteria-containing mixture was infunded to the flat plate and allowed to solidify. In each plate, three pores (4 mm) were dug in aequalis distribution by sterile perforex and 50 μl curcumin analogs in different concentrations were poured into a pore. Antibiotic ampicillin, curcumin, and DMSO were used for comparison in the same method. The flat plates were incubated at 37°C for 24 h under 5% CO_2 in the upright position. The diameter of the zone of inhibition was measured using scale [22,23].

Acknowledgements

This work was supported by the general grant of Zhejiang administration of Chinese Traditional Medicine (2007CA080 and 2007CA079), Wenzhou key research project (S2005B001), and research project of Wenzhou Medical College (89007046), and also by the Zhejiang Department of Education science grant (20070992).

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